Variations in Hematologic Responses to Increased Lead Absorption in Young Children

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In the study of human populations, much emphasis is placed on the concentration of lead in whole peripheral blood. There is a considerable body of evidence which indicates that this measurement reflects recent and current assimilation of lead. While broad ranges in blood lead concentration have been associated with differing risks of toxicity for groups, it is not a precise index of adverse effect per se, even at elevated levels. Within the red blood cell itself there is not a close association between the concentration of lead and such adverse metabolic effects as the increased loss of potassium caused by lead. Above the apparent "threshold zone" of approximately 30–50 μg Pb/100 ml whole blood, equivalent metabolic effects on heme synthesis may be seen over an interval of at least 20 μg Pb/100 ml whole blood. This variation will be examined with particular reference to the interrelationship between the concentrations of lead and protoporphyrin in peripheral blood. The data indicate that limitations in both precision and accuracy of measurement account for a relatively small fraction of the observed variations. Together with other experimental and clinical information, they suggest that concurrent dietary deficiency of iron may be one of the important modifying factors in the responses of subjects with increased lead absorption. It is suggested that suspected adverse effects upon the various organ systems associated with increased lead absorption be measured directly and that the CaEDTA mobilization test for lead should be more fully explored as a measure of the "metabolically active" fraction of the total body lead burden.

Introduction

In the study of human populations, much emphasis is placed on the concentration of lead in whole peripheral blood (μg Pb/100 ml whole blood). There is a considerable body of evidence which indicates that this measurement reflects the level of recent and current assimilation of lead (*1*). Followup studies show that slight to moderate elevations are also found in individuals with a history of "high dose" types of exposure in the remote past (*2*). In short, the measurement is affected by the balance among assimilation, excretion, and storage, but strictly speaking, it is not a measure of effect. While broad ranges in blood lead concentration are associated with differing risks of toxicity, studies in individuals make it clear that this measurement is not a precise index

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of toxic effect, *per se*, even at elevated levels. Above the apparent "threshold zone" of approximately 30–50 μg Pb (*1*), equivalent metabolic effects on heme synthesis may be seen over a span of at least 20 μg Pb. Often—and sometimes with good reason—the apparent lack of close correlation between blood lead concentration and various effects is ascribed to imprecise laboratory data (*3*). However, interpretations based on biological and epidemiological variables also deserve careful consideration. For example, within the red blood cell itself, there is no close association between the total concentration of lead and such adverse effects as the increased loss of potassium caused by lead (*4, 5*). There is also some experimental evidence that certain nutritional deficiencies (*6, 7*) may interact with lead and so modify the effects observed. The interplay between absorption and effect may be examined more closely by using the interrelationship between the hematocrit and the concentrations of lead and erythrocyte protoporphyrin (PROTO) in peripheral blood as an example.

Methods and Materials

In late 1971, in collaboration with the Bureau of Community Environmental Management, HSMHA, DHEW, 115 presumably well, preschool children from old housing areas in Charleston, S.C. were tested. Hematocrit and protoporphyrin were measured in duplicate capillary blood samples. Venous blood was drawn simultaneously for lead determination. Protoporphyrin was measured immediately by a one-step extraction technique, as described elsewhere (*8*). Lead was measured in dilute perchloric acid filtrates of whole blood by atomic absorption spectrophotometry (AAS) at 283.3 nm, simultaneous deuterium background correction and similarly treated normal human blood spiked with lead being used as reference standards to compensate for nonspecific matrix effects (Chisolm and Harrison, in preparation). Standard statistical techniques, including least-squares regression and analysis of covariance, were used.

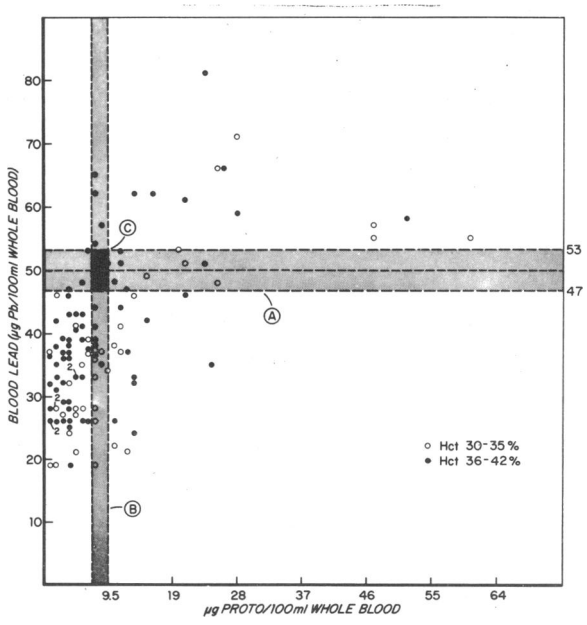

FIGURE 1. Relationship between concentration of lead and protoporphyrin in whole blood in preschool children: (○) children with 30–35% hematocrit; (•) children with 36–42% hematocrit. Bands A and B show precision limits for each type of measurement (see text).

Results

The results of this study are shown in Figure 1. Although both lead and protoporphyrin are found almost exclusively in or on red blood cells, the data are presented in terms of their concentrations in whole blood, the medium in which each was measured. For the acidified acetone method used to assay protoporphyrin, the "normal" value is 3.17 ± 1.36 μg PROTO/100 ml whole blood or 7.58 ± 3.25 μg PROTO/100 ml red blood cells for 38% hematocrit. This normal value is about one-seventh of the values found by classical extraction methods for free erythrocyte protoporphyrin (FEP). It was determined in a separate group of 34 preschool children who were carefully selected to exclude anemia (hematocrit $\geq$ 36%) and even minimal inhibitory effects of lead on heme synthesis (whole blood lead concentrations of $<$ 30 μg Pb by two independent methods of analysis). The mean for this group was 20.7 ± 5.4 μg Pb. The reliability of the PROTO assay was determined in a separate

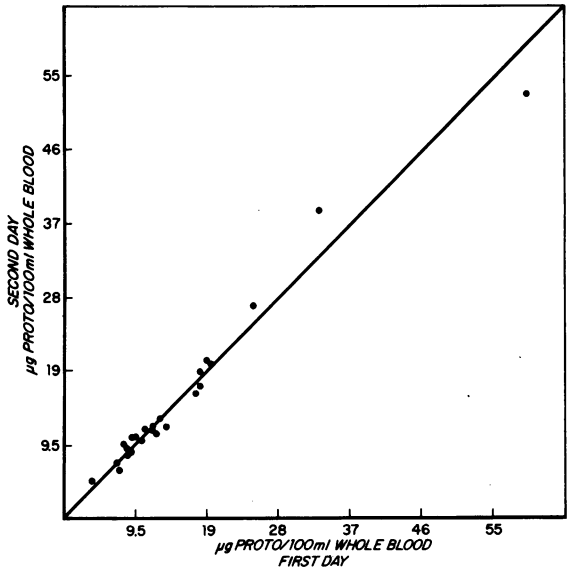

FIGURE 2. Comparison of protoporphyrin values obtained in 24 children on two successive days. For each child, the first day's result is plotted on the abscissa against the second day's result on the ordinate. The diagonal line represents the line of perfect positive correlation. For the actual data, the slope of regression does not differ significantly from 1, the intercept does not differ significantly from 0 and $r = 0.98$.

group of 24 children in whom the assay was repeated on successive days on coded samples. The first day's result is plotted against the second day's result in Figure 2. The test shows good day-to-day reproducibility in individual children. In Figure 1, a curvilinear relationship between the concentrations of lead and protoporphyrin in blood is apparent. For the group as a whole, the relationship between blood lead concentration and ln [PROTO] is linear, statistically significant ($P < 0.001$, $r = 0.60$) and is described by the following equation: μg Pb = 22.30 + (8.92 ln [PROTO]) S.D. = $10.04_{y/x}$. Above the apparent threshold zone of 30–50 μg Pb, there is great variation in the response of individuals as measured by PROTO. To illustrate the portions of the observed differences attributed to limitations in the precision of the measurements, hatched areas A, B, and C have been superimposed on Figure 1. Band B shows the range of difference between duplicate PROTO measurements (2.26 μg PROTO/100 ml whole blood) at a concentration level which is just above the normal range. Band A shows the 95% confidence limits for the precision of the AAS method for blood lead analysis and is superimposed at the 50 μg Pb level. Area C represents the intersection of bands A and B and shows the approximate 95% confidence limits for the measurements at the 50 μg Pb level. The "relative accuracy" of the lead method was studied in a separate group of 113 children with blood lead levels in a comparable concentration range in whom venous samples were split for measurement by both the AAS and a double extraction dithizone technique. Least-squares regression analysis indicates that the two methods give comparable results (*9*). No significant proportional error was found (slope = 1.022), constant error was small (intercept = 2.0 μg Pb), but the random error using *N*-2 was $\pm$3.7 μg Pb ($S.D._{y/x}$). Similarly, in split samples of blood, it was found that the acidified acetone method gives values for protoporphyrin which in heparinized blood are one-seventh of the values found with a double extraction technique (*8*) for free erythrocyte protoporphyrin. It does not appear that errors in measurement of the order of 10% account for the 400% to 800% variations found in PROTO in children with elevated blood lead levels.

The 115 children in the group were divided into two subgroups according to hematocrit: 40 had hematocrits of 30–35 and 75 had hematocrits of 36–42%. Least-squares regression analysis revealed two separate, but essentially parallel slopes in each subgroup for the regression of blood lead concentration on protoporphyrin concentration (Fig. 3). Covariance analysis showed that these regression lines differ significantly at the 0.01 probability level. Throughout the range of blood lead concentration, those with 30–35% hematocrit show PROTO values which are double the values found in the children with 36–42% hematocrit. The data suggest that mild degrees of anemia account for at least a part of the differences in response

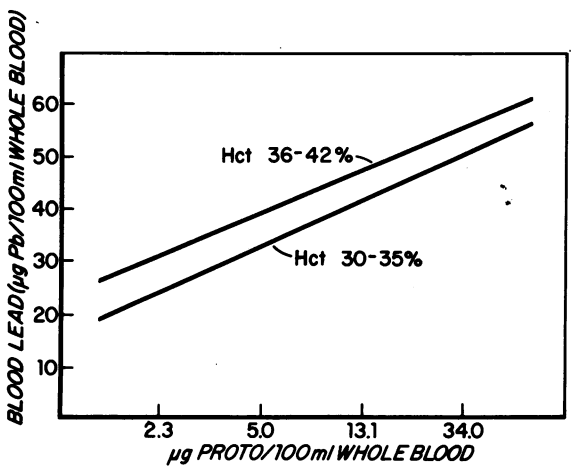

FIGURE 3. Relationship between concentrations of lead and protoporphyrin in whole blood according to hematocrit subgroupings. Separate but essentially parallel slopes which differ significantly at the 0.01 probability level are found for normal and slightly reduced hematocrit ranges in the regression of PROTO on blood lead concentration. For the two groups, the equations are as follows: For 36–42% (mean = 38.2%) hematocrit: μg Pb = 24.16 + (9.06 ln [PROTO] and $S.D._{y/x}$ = 9.82, N = 75, r = 0.59, $P<0.001$, range of blood lead concentration = 19–81 μg Pb (m = 40.7μ g Pb). For 30–35% (mean = 33.9%) hematocrit: μg Pb = 16.22 + (9.95 ln [PROTO] and $S.D._{y/x}$ = 9.41, N = 40, r = 0.70, $P<0.001$, range of blood lead concentration = 19–71 μg Pb (m = 36.9 μg Pb).

which were found in children with the higher blood lead concentrations.

Discussion

These results confirm the reports of others (*10–12*) who also found a curvilinear relationship between the concentrations of lead and protoporphyrin in blood. This report extends these observations by pointing to an interaction between lead absorption and mild anemia. The variation in metabolic response observed is not unique to this particular interrelationship. The report of Selander and Cramer (*13*) is representative of a number of studies. They found a similar curvilinear relationship between blood lead concentration and ALA excretion. At levels between 40 and 70 μg Pb, they found large differences in ALA excretion ranging between normal and five times greater than normal. Similarly, we have found in children that there is a statistically significant semilogarithmic relationship between blood lead concentration and the response to the CaEDTA mobilization test; however, there is considerable variation about the line of regression, even when timed quantitative 24-hr urine collections are made (Chisolm, unpublished data).

It is difficult to arrive at firm conclusions on the basis of data obtained at a single point in time, as was the data just presented. The dynamic longitudinal aspect of the metabolism of lead and variables in individuals may not be evident. In addition, the clinician deals with unknown and uncontrolled variables, rather than the "steady state" of the experimentalist. Nevertheless, these data may be interpreted in the light of other work and at least to the extent of pointing out fruitful directions for future inquiry. Among the 115 children whose data are shown in Figure 1, there were nine with borderline elevations in protoporphyrin concentration (7–14 μg PROTO/100 ml whole blood) who had blood lead concentrations of 51–65 μg Pb. None were anemic (37–42% hematocrit) and, save one three-year-old, all were 4 or 5 years of age. These discrepancies may reflect recent large, but isolated ingestions of lead. Precedent for this interpretation is found in the balance studies of Kehoe (*14*) who observed transient increases in blood lead concentration of similar magnitude in association with transient increases in the alimentary intake of lead. Similarly, recent studies in baboons (*15*) suggest that single large doses of lead may be cleared rapidly from the blood. We now have serial measurements in several children which are consistent with this hypothesis; namely, stable borderline increases in PROTO have been observed in association with blood lead concentrations which occasionally spike to 60–65 μg Pb from a basel level of 35–45 μg Pb. Alternatively, since eight of the nine children cited were 4 or 5 years of age, the findings may represent the influence of the slow excretion of excess boney stores of lead resulting from prior increased intake, rather

than brief excessive current intake. There is also precedent for this interpretation (*2, 14, 16*). Only serial measurements can aid in choosing among these alternatives; however, despite their elevated blood lead levels in the 51–65 μg Pb range, it is clear that these nine children were not showing any obvious untoward hematologic effects: their hematocrits were normal and their PROTO values fell just above the normal range. This may be contrasted with protoporphyrin values reported in children with manifest plumbism which were 40–250 times normal levels (*17*).

There were 15 other children in this group with blood lead concentrations in the 50–80 μg Pb range in whom PROTO values ranged between 5 and 45 times the normal values; these are the ones showing the greater adverse metabolic effect. Statistical treatment of the data suggests that mild reduction in hematocrit is a significant factor interacting with lead in this group. Although more extensive hematologic data were not obtained, epidemiologic considerations (*18*) and experimental data (*1, 6*) strongly implicate iron deficiency as the factor most likely to account for the marked difference in metabolic effect observed. Data in adults suggest that erythrocyte protoporphyrin is, perhaps, the most sensitive index of both latent and manifest iron deficiency (*19*).

In the inherently unsteady clinical situation in which intake of lead by children may fluctuate widely in relation to variable residence and multiple caretakers, direct measurements of various effects such as erythrocyte protoporphyrin may provide a valuable index of subclinical effect as well as average assimilation over a period of time. It would be of interest to know how closely effects on heme synthesis are related to the effects of lead in other target organs such as the nervous system. In one pediatric study in which subjects and controls were separated on the basis of residence and coproporphyrinuria, measurable deficits were found on followup in fine motor function and behavior (*20*). It is entirely possible that the subclinical effects of plumbism in the nervous system and the hematopoietic system may be more closely related to one another than either is to infrequent measurements of blood lead concentration which may be acutely affected by sharp fluctuations in assimilation. Concurrent anemia—even of mild degree—and quite possibly other factors should also be taken into account in evaluating the effects of increased lead absorption.

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